IBC protocol Risk Assessment and Determination of *NIH Guidelines*

The following are points to consider when reviewing all protocols for risk, recommended containment conditions, and determine applicable NIH Guidelines.

Risk Assessment Overview:

The risk assessment framework of the *NIH Guidelines* uses the risk group of the parent organism as a starting point for determining the necessary containment level. For example, genetic modifications of a Risk Group 3 organism would generally be carried out at Biosafety Level 3 (BSL-3) containment but the containment level might be raised or lowered depending on the specific construct and the experimental manipulations.

In most instances, this risk assessment framework can be effectively applied to assess the biosafety risks of experiments with synthetic nucleic acids. However, synthetic biology research has the potential to create complex, novel organisms for which identification of a parent organism may be more difficult or may not be as relevant to the risk assessment as it is with more traditional recombinant organisms. The risk assessment may also be complicated by the limitations in predicting gene function from sequence(s) or the synergistic effects from combining sequences from different sources in a novel context. In such cases, the risk assessment should include at least two levels of analysis. The first involves a consideration of the Risk Groups of the source(s) of the sequences and the second involves an assessment of the functions that may be encoded by these sequences (e.g., virulence or transmissibility). It may be prudent to first consider the highest risk group classification of all agents that are the source of sequences included in the construct. Other factors to be considered include the percentage of the genome contributed by each parent agent and the predicted function or intended purpose of each contributing sequence. The initial assumption should be that all sequences will function as they did in the original host context.

The risk assessment should also consider that the combination of certain sequences in a new biological context may result in an organism for which the risk profile could be higher than that of the contributing organisms or sequences. The synergistic function of these sequences may be one of the key attributes to consider in deciding whether a higher containment level is warranted, at least until further assessments can be carried out. A new biosafety risk may occur with an organism formed through the combination of sequences from a number of organisms or due to the synergistic effect of combining transgenes that results in a new phenotype.

Elements to consider when assessing risk:

- **Agent or Vector:**
    - Is agent/vector from Risk group: RG-1, RG-2, RG-2, RG-4, Select Agent (SA)
    - Infectious material, pathogen, opportunistic pathogen, biological toxin, human/NHP body fluid, cells, or tissue
    - Host range of agent/ vector: human, broad/multi-host, environmentally or agriculturally important
    - Can an attenuated strain or killed organism be used
    - Characteristics of agent/vector: spore former, exotic agent, hard to kill or easy to acquire, low infectious dose, attenuated strain, killed organism
    - Mode of transmission: aerosol/fomite
    - Lab procedures with risk of exposure: vortexing, centrifugation, sharps, needles or injection, animals

- Quantity and/or concentration of agent/vector made or used in work, purification method
- Prophylaxis or treatment available or recommended
- Viral vector:
  - Source: commercial, collaborator, lab designed
  - host range: xenotropic, amphotropic (envelope/pseudoytpe)
  - vector production: provided as complete vector or describe including packaging cell line
  - safety features: genome split in multiple plasmids, deleted structures
  - is it replication competent virus: modifications, has it been tested

- **Host:**
  - Animal used in any part of the research:
    - Species: rodent, fish, insect, etc
    - Transgenic or creating new strains
    - Permissive species: humanized, immune deficient, or carry endogenous adventitious agents, viruses, or sequences such as gammaretroviral LTR
  - Animals used as host:
    - Viral vector or infectious agent challenge/exposure
    - Will exposure to the infectious agent/viral vector pose a risk of infecting other animals; Horizontal versus vertical transmission
    - Used for xenograft, allograft, transplant, or tumor studies
    - Will the ability to control the host(e.g., insects) be modified: treatment by pesticides/insecticides,
  - Cell culture used in any part of the research:
    - Human cells, non-human primate cells, stem cells, any primary culture
    - Transformed, transfected, or cancer (tumor) cell line
    - Cells containing endogenous adventitious agents, viruses, or viral sequences
    - Host for expression system, virus packaging cell line, or virus propagation
    - Used *in vitro* or *in vivo* for transplant/allograft/xenograft studies
  - Insect cell lines used:
    - Baculovirus vector
  - Plant hosts used in any part of research:
    - *Agrobacterium* vector
    - Potential agricultural or ecological impact from plant host, agents used including recombinant, Tg plant
    - Noxious weed as host
    - Transgenic plant allowed to reproduce or have ability to interbreed with noxious weed
    - Virus with obligate insect vector
    - Sequences encoding potent vertebrate toxins introduced in plants or associated organisms
    - Will the ability to control the host be modified i.e. treatment by herbicides
  - Bacteria, fungi, virus, parasitic agent, or other microorganism used as host:
    - Is host from RG-1 such as *E. coli* K-12 strains, *S. cerevisiae*, *S. unarum*, *Kluyveromyces*, or asporogenic strains of *B. subtilis* or *B. licheniformis*
    - Is host an opportunistic pathogen, RG-2, RG-3,or RG-4 agent, Select Agent, or agriculturally significant
    - Will the virulence or pathogenicity of host be modified
    - Will a toxin be produced
    - Will this effect medical treatment for this agent
    - Can a surrogate organism or attenuated strain be used

- **Genes:**
    - Is the gene or sequence (including synthetic) from RG-2, RG-3, or RG-4 agent, biological toxin, or Select agent
    - Any risks associated w/ the gene or sequence such as: up-regulation/silencing expression, regain of function, oncogenes, virulence factors, toxins, or expanded host range
    - Does the gene or sequence change sensitivity to antibiotics, herbicides, pesticides, or insecticides that would be used to control the host

NIH Guidelines and Biocontainment:

- If protocol involves recombinant or synthetic nucleic acid molecules (DNA, RNA, synthetic) determine which section(s) of the *NIH Guidelines* apply. Most work will fall under sections III-D, III-E, or III-F. If it is a low risk BSL-1 protocol that doesn't really fit under III-D or III-F, it is covered under III-E.
- *NIH Guidelines* Sections III-D, III-E, and III-F specify minimum containment, but may also allow the IBC latitude to set or modify containment conditions with consideration of the risk assessment.
- Biocontainment conditions for the laboratory setting and animal work based on the *BMBL* nomenclature are: BSL-1, BSL-2, BSL-3, BSL-4 and ABLS-1, ABSL-2, and ABSL-3. Use designations of BSL-1 and BSL-2 for animals such as fish and insects
- Work done with human and non-human primate cells should be done using BSL-2/ABSL-2 containment
- Biocontainment for plants are BL1-P, BL2-P
- Large scale quantities (>10 liter vessel) BL1-LS, BL2-LS
- Base risk assessment on: 1) Risk Group of agent or risks associated with agents and genes, 2) what will be manipulated and how, 3) quantity or concentration recombinant materials or vectors, 4) source or genes and/or vectors, 5) procedures and equipment used in work, 6) facilities, 7) PPE, and 8) training, and experience of the personnel

Determining Applicable protocol designations under the *NIH Guidelines:*

- **Infectious Agents Section III-D-1**: protocol using RG-2, RG-3, RG-4 agent as Host-Vector Systems. See Section III-D-7 if using Influenza virus
- **Cloning DNA from Infectious Agents Section III-D-2:** protocol using recombinant/synthetic nucleic acid molecules from RG-2, RG-3, RG-4 or Select Agent cloned into non-pathogenic prokaryotic or lower eukaryotic Host-Vector Systems, unless DNA codes for biotoxin toxic for vertebrates in Section III-B
- **Viral Vectors in Cell Culture Section III-D-3**: protocol using infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of a helper virus in Tissue Culture Systems
- **Viral Sequences in Cell Culture Section III-E-1 or III-F-8**: protocol using recombinant/synthetic nucleic acid molecules containing <½ to $\leq^2/_3$ eukaryotic viral genome respectively propagated and maintained in Tissue Culture Systems (no helper virus involved)
- **Cloning DNA using RG-1 Host** (RG-1 Host-Vector System) **Section III-E or III-F-8:** protocol using recombinant/synthetic nucleic acid molecules from RG-1 agent cloned into non-pathogenic prokaryotic RG-1 agent. See Appendix C for Host-Vector Systems qualified as low risk/exempt.
- **Transgenic Animals Sections III-E-3, III-D-4, or III-F-8:** protocol involves the creation or use of transgenic animals, including the introduction of recombinant/synthetic nucleic acids, genetically modified organisms, or genetically modified cells.
- **Transgenic Plants Sections III-D-5 or III-E-2:** protocol involves the creation or use of transgenic plants including algae, or the introduction of recombinant/synthetic nucleic acids or genetically modified organisms

- **More than 10 liters of Culture Section III-D-6:** protocol involves the large scale (>10 liter vessel) culture of genetically modified organisms
- **Influenza Viruses Section III-D-7:** protocol involves influenza viruses generated by recombinant or synthetic methods (e.g., generation by reverse genetics of chimeric viruses with reasserted segments, introduction of specific mutations)
- **Transfer of Drug Resistant Trait Section III-A:** protocol involves the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally if such acquisition could compromise the use of the drug to control disease agents in humans veterinary medicine, or agriculture applies. ** requires approval from IBC, NIH/OBA, may also require RAC review and NIH Director approval*
- **Toxins Section III-B:** protocol involves recombinant/synthetic nucleic acids containing genes for the biosynthesis of a biotoxin with vertebrate $LD_{50} \leq 100$ng/kg BW (e.g., botulinum toxins, tetanus toxin, diphtheria toxin, and S. dysenteriae neurotoxin), *requires review and biocontainment set by NIH OBA. Or cloning in *E. coli* K-12 strains, segments for the biosynthesis of toxin molecules with vertebrate LD_{50} between 100 ng/kg BW and 100 □g/kg BW may be included in specific experiments already approved by NIH OBA under this section but **requires consultation with NIH OBA.*
- **Humans Section III-C:** protocol involves the deliberate transfer into human research participants (human gene transfer) (A) recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic molecules, or (B) synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules that meet any of the following criteria: 1- contains > 100 nucleotides; or 2- possesses biological properties that enable integration into the genome (e.g., cis elements); or 3- have the potential to replicate in a cell; or 4-can be translated or transcribed **requires IBC, IRB approval and NIH OBA RAC review. No human gene transfer work currently conducted at UT*

Qualified low risk/exempt research Section III-F: protocol using recombinant or synthetic nucleic acids that:

- **Section III-F-1** synthetic nucleic acid molecules that: (A) can neither replicate or generate nucleic acids that can replicate in any living cell such as oligonucleotides or synthetic nucleic acids that do not contain origin of replication or elements known to interact with DNA or RNA polymerase, (B) are not designed to integrate into DNA, and (C) does not produce a toxin with $LD_{50} < 100$ng/kg BW. (D) not transferred into humans
- **Section III-F--2** not in organisms, cells or virus,
- **Section III-F-3** consist solely of the exact recombinant/synthetic nucleic acid sequence from a single source that exists contemporaneously in nature
- **Section III-F-4** consisting entirely of DNA from a prokaryotic host propagated in the same or closely related strain,
- **Section III-F-5** consisting entirely of DNA from eukaryotic host (except viruses) propagated in the same or closely related strain of the same species. Includes breeding transgenics with only genes from that species manipulated; no foreign sequences.
- **Section III-F-6**: using DNA segments from different species that naturally exchange DNA, see Appendix A
    - **Section III-F-7:** using DNA molecules that have acquired a transposable element that does not contain recombinant and/or synthetic DNA.
    - **Section III-F-8:** using A) qualified low risk/exempt Host-Vector Systems, B) extrachromosomal elements, or C) purchase, transfer, and breeding of ABSL-1 transgenic rodents. See Appendix C for conditions and exemptions

Risk Assessment and Biocontainment for research (not recombinant) with infectious or other potentially infectious material (OPIM) and/or biological toxins include:

- Infectious or OPIM includes pathogens, human and non-human primate cells, tissue, or fluids, and may also include opportunistic pathogens. Possible containment conditions include: Universal Precautions, BBP safety practices, BSL-2 or higher, and ABSL-2 or higher, and OHP vaccination recommendations
- Biological toxins including toxins not listed as SA, exempt quantities, and with LD_{50} < 1mg/kg BW. Possible containment conditions include BSL-1 or higher with biotoxin safety practices and/or select agent requirements, and OHP vaccination recommendations
- Base risk assessment on: 1) Risk Group of agent or risks associated with agents or materials used, 2) how will it be used, 3) quantity or concentration, 4) source, 5) procedures and equipment used in work, 6) facilities, 7) PPE, and 8) training, and experience of the personnel
- Biocontainment conditions are similar to those used for recombinant work

References for Risk Assessment: *NIH Guidelines*: Section II-A and Appendix A, B, C, and E. *BMBL*: Sections I, II, and VIII, and Appendix D, F, and H. *NIH Guidelines* Section II provides guidance for performing a comprehensive risk assessment and determining the appropriate containment conditions for protocols submitted for review. Additional resources referenced are: the *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th ed.* and the Occupational Safety and Health Administration (OSHA) regulation, 29CFR 1910.1030 and OSHA publication 3127.

References for Physical and Biocontainment Conditions: *NIH Guidelines*: Sections III-D, III-E, and III-F have work specific minimum containment conditions and described in Appendix C, F, G, I, K, P and Q. *BMBL*: Sections III, IV, and V and Appendix A, E, and I.